A Prospective Study of Unfractionated Heparin Therapy in Dogs With Primary Immune-Mediated Hemolytic Anemia

Unfractionated heparin therapy was initiated at a standard dosage of 300 IU/kg subcutaneously *q* 6 hours to 18 dogs with immune-mediated hemolytic anemia. Heparin's prolongation of activated partial thromboplastin time and change in factor Xa inhibition (anti-Xa activity) were serially monitored during the first 40 hours of therapy. During the initial 40 hours, only eight of 18 dogs had attained anti-Xa activities of ≥0.35 U/mL. No dogs had clinical signs of hemorrhage. Fifteen dogs survived to discharge; 11 dogs were alive at 1 year, and thrombosis was identified in three of six nonsurvivors that were necropsied. **J Am Anim Hosp Assoc 2009;45:125-133.**

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Introduction

Thromboembolic events are a common complication and an important cause of mortality in dogs with immune-mediated hemolytic anemia (IMHA). Thromboembolic events have been documented at necropsy in 11% to 80% of dogs with IMHA.[1-5] Laboratory abnormalities, such as thrombocytopenia, hyperbilirubinemia, leukocytosis, and hypoalbuminemia, have been associated with development of thromboembolism in dogs with IMHA; however, the pathogenesis of thrombus formation and effective regimens for thromboprophylaxis have not been established.[1,3-5]

The concept of "Virchow's Triad" (endothelial damage, alterations in blood flow, and hypercoagulability) refers to the underlying factors that promote arterial and venous thrombus formation in various disease states.[6,7] The relative importance of each factor in IMHA-associated thromboembolism is unknown; however, hemostatic abnormalities consistent with hypercoagulability are common in dogs with IMHA.[1,8,9] The reported high plasma fibrinogen, soluble fibrin, and D-dimer concentrations in dogs with IMHA are indicative of an excess of thrombin substrate and the unopposed systemic action of thrombin on fibrinogen to generate cross-linked fibrin. Soluble fibrin monomer is an intermediary product produced by thrombin's cleavage of fibrinogen. The specific fibrin degradation product, D-dimer, is produced only subsequent to this action of thrombin on fibrinogen, followed by thrombin-activated factor XIIIa cross-linkage of fibrin polymer. The findings of high-soluble fibrin and high D-dimer are therefore direct and indirect indicators of thrombin's action on its substrate, fibrinogen. The concomitant finding of low antithrombin activity in dogs with IMHA provides further evidence of hemostatic imbalance favoring coagulation and subsequent fibrin deposition.[1,8,9]

Unfractionated heparin (UFH) is often administered empirically to dogs with IMHA in an attempt to inhibit coagulation and prevent thromboembolism; however, few clinical studies have evaluated the efficacy of UFH for reducing morbidity or mortality caused by thrombosis in this population. In human trials of UFH thromboprophylaxis, laboratory monitoring is performed to gauge UFH's anticoagulant effect for individual patients. The clinical efficacy of UFH is optimized by attaining a minimum level of anticoagulant intensity, but the risk of bleeding

increases if the therapeutic range is exceeded.[10,11] Determination of clotting time in the activated partial thromboplastin time (aPTT) screening test is a common method of UFH monitoring, with dosing to attain a target prolongation of 1.5 to 2 × the patient's baseline or control aPTT value.[10,11] Alternatively, UFH biological activity can be monitored based on the *in vitro* inhibition of an activated factor X (factor Xa) reagent. Factor Xa inhibitory assays (i.e., anti-Xa activity assays) are not influenced by many of the patient and assay variables that affect the aPTT, and they have been used in pharmacokinetic and pharmacological outcome studies of UFH therapy in dogs.[12,13] In human trials, a target range of anti-Xa activity from 0.35 to 0.70 U/mL is generally considered effective for thromboprophylaxis.[14-19] Target therapeutic ranges for UFH associated with improved clinical outcomes have yet to be established for any thrombotic syndrome in dogs. Furthermore, laboratory monitoring of UFH using anti-Xa activity assays has not been investigated in dogs with IMHA.

The objective of this pilot study was to determine whether dogs with IMHA given a subcutaneous (SC) dosage of 300 IU/kg UFH *q* 6 hours would attain anti-Xa activity levels in the range of 0.35 to 0.70 U/mL. The target range of 0.35 to 0.70 U/mL anti-Xa activity was based on extrapolation from human UFH pharmacological outcome studies.[17,18] The IMHA cases were enrolled at a single referral hospital, with serial monitoring of aPTT and anti-Xa activity during the first 2 days of UFH therapy. Outcome measures included attainment of target range anti-Xa activity within 40 hours of initiating UFH, clinical evidence of bleeding, necropsy evidence of thrombosis or hemorrhage, survival to discharge, and survival at 1 year.

Materials and Methods

All dogs in this study were cases at the Purdue University Veterinary Teaching Hospital. Dogs were enrolled after owner consent was received over a 22-month period (June 2003 to March 2005). The criteria used for diagnosis of primary IMHA were a hematocrit of ≤25% (37% to 55% reference interval); presence of spontaneous persistent agglutination (that persisted after dilution with saline) or at least 2+ spherocytes on a blood smear; and no evidence of an underlying disease or history of drug administration that could cause secondary IMHA. Spherocytes were reported as 3+ if 51 to 150 spherocytes were noted per 1000× field, 2+ if 11 to 50 were noted per 1000× field, and 1+ if 1 to 10 were noted per 1000× field. Spherocytes were reported as occasional if they were seen in some 1000× fields. Ten or more high-power fields were evaluated for each count.

Prior treatment with corticosteroids did not preclude a dog's enrollment; however, administration of corticosteroids could not exceed 7 days. Dogs were excluded from the study if they had received prior treatment with cytotoxic drugs (i.e., azathioprine, cyclophosphamide, cyclosporine) or anticoagulants (i.e., heparin, warfarin). Prior treatment with blood products (e.g., plasma, packed red blood cells [pRBCs], whole blood) did not exclude dogs from the study;

however, a diagnosis of IMHA needed to have been made prior to blood transfusion.

To confirm a diagnosis of primary IMHA, the following tests were performed: complete blood count (CBC) with evaluation of red blood cell (RBC) and white blood cell morphology, platelet count, reticulocyte count, direct Coombs' test, serum biochemical profile, urinalysis, prothrombin time (PT), aPTT, infectious disease titers (i.e., *Ehrlichia canis*, *Rickettsia rickettsii*, *Babesia canis*), thoracic and abdominal radiographs, ultrasonographic examination of the abdomen, and cytological evaluation of a bone marrow aspirate (if anemia was nonregenerative). The direct Coombs' test was performed at 37°C using a combined Coombs' reagent containing goat anti-canine immunoglobulin G, immunoglobulin M, and complement component C3.[a] Erythrocytes were washed four times prior to addition of the Coombs' reagent.

Treatment Protocol

All dogs were treated with UFH[b] at an initial dosage of 300 IU/kg SC *q* 6 hours. This dosage was based on preliminary findings in three dogs with IMHA (not included in this study) that received a starting dosage of 200 IU/kg SC *q* 6 hours. None of these dogs attained target-range anti-Xa activity within 40 hours of initiating UFH (40-hour anti-Xa values of <0.1, 0.18, and <0.1 U/mL); therefore, the initial dosage was increased in this study in an attempt to achieve anti-Xa activity in the target range. All dogs were treated with prednisone at 2 mg/kg per os (PO) *q* 12 hours or at one-seventh of the dosage if using dexamethasone parenterally.[20,21] A lower dosage of prednisone (but not <20 mg/m² *q* 12 hours) could be used in dogs weighing >35 kg. Azathioprine (2 mg/kg PO *q* 24 hours) or cyclophosphamide (50 mg/m² intravenously [IV] *q* 24 hours for four doses; then 3 days off prior to resuming daily treatments) was added if no increase in the hematocrit was seen after 10 to 14 days of treatment or if two or more transfusions (blood or hemoglobin-based oxygen carrier) were required. All dogs received famotidine (0.5 mg/kg IV or PO *q* 12 hours). Packed red blood cells, hemoglobin-based oxygen carriers, or antibiotics were administered at the discretion of the attending clinician.

Sample Collection

Blood samples for coagulation assays and anti-Xa activity were collected directly into plastic syringes containing 3.8% sodium citrate anticoagulant. The total sample volume was 4.0 mL, and the volumes of blood and citrate were adjusted to correct for anemia as follows: 0.4 mL citrate and 3.6 mL blood for dogs having a hematocrit of >29%; 0.5 mL citrate and 3.5 mL blood for dogs having a hematocrit of 15% to 29%; and 0.6 mL citrate and 3.4 mL blood for dogs having a hematocrit <15%. This volume adjustment produced a final concentration of 0.64% citrate in the supernatant plasma.[1]

Following collection, blood samples were centrifuged at 3000G (3000× the force of gravity) for 15 minutes at room temperature. The plasma was then aspirated and immediately

frozen at –20˚C in plastic tubes.[22] After collection of the 40-hour sample, all samples were shipped to the Cornell Comparative Coagulation Laboratory overnight in a container with dry ice. A plasma sample from a healthy dog was included as a shipping control with each batch of IMHA samples, to allow for detection of deterioration of sample quality during transit. The finding of fibrinogen deficiency or prolongation of clotting time for the control sample would be considered evidence of suboptimal transit and preclude evaluation of the patient samples.

The starting time of UFH administration was standardized to ensure follow-up samples were collected at the appropriate intervals. Subcutaneous UFH was started at 8 AM, 2 PM, 8 PM, or 2 AM and was continued every 6 hours at the initial dose until any adjustments were made. Follow-up samples were collected (in the same manner as the initial, pretreatment sample) at 4, 16, 28, and 40 hours after UFH administration was instituted (each sample was drawn at 4 hours after a UFH dose to evaluate peak plasma concentrations).[13,23]

Laboratory Assays

A coagulation panel consisting of aPTT, PT, fibrinogen concentration, D-dimer concentration, and antithrombin activity was performed on all pretreatment samples (time 0 hour). At the 4-, 16-, 28-, and 40-hour time points after initiating UFH, aPTT and anti-Xa activity were measured. Antithrombin activity was also measured at the 40-hour time point. All coagulation testing was performed at the coagulation laboratory within 24 hours of receiving the samples. An additional aPTT was performed at Purdue University Veterinary Teaching Hospital at the 40-hour time point or at any other time when there was concern about the risk of excess anticoagulation.[c] In dogs that were monitored beyond the 40-hour period, aPTT and anti-Xa activity were measured 48 hours after each dose increase. In dogs discharged for home administration of UFH, aPTT and anti-Xa activity were measured at each recheck examination.

Coagulation assays (i.e., aPTT, PT, fibrinogen) were performed in singlet using commercial reagents[d,e,f] and using an automated coagulation instrument[g] with a mechanical endpoint method, as previously described.[1,22,24] Plasma concentration of D-dimer[h] was measured using commercial latex agglutination kits, according to the manufacturers' specifications. Antithrombin activity was measured using a chromogenic substrate kit[i] in a test procedure that is insensitive to therapeutic levels of UFH.[25] The antithrombin values of IMHA dogs were reported as a percentage of a pooled normal canine reference plasma (prepared from 20 healthy dogs) having an assigned value of 100% antithrombin.

The plasma UFH content, based on factor Xa inhibitory activity of UFH, was measured in an amidolytic, one-step competitive inhibition assay,[j] modified by use of a higher sample dilution as previously described.[22] The assay is configured with a bovine factor Xa reagent and chromogenic substrate of factor Xa, and it is used without the addition of exogenous antithrombin.[k] The assay's threshold of detection for UFH anti-Xa activity is 0.1 U/mL.[22] The recovery of UFH activity is not influenced by a freeze-thaw cycle, and the expected interassay coefficient of variation for samples containing 0.5 U/mL UFH is <10%.[22]

Adjustment of UFH Dosage

In dogs that survived beyond 40 hours, further dosage adjustments were made based on the 40-hour anti-Xa activity. If anti-Xa activity fell below target range (<0.35 U/mL), the UFH dosage was increased by 17% to 25%. If anti-Xa activity was within target range (0.35 to 0.70 U/mL), no dosage adjustment was made. If anti-Xa activity rose above the target range (>0.70 U/mL), the UFH dosage was decreased by 25% to 50%. During the first 40 hours of UFH treatment, dogs were monitored daily for any evidence of hemorrhage; frequent physical examinations were conducted by the principle investigators, and packed cell volume and total protein were measured at least twice a day. For dogs that were discharged, owners were instructed to monitor oral mucous membrane color and look for any evidence of hemorrhage (e.g., cutaneous, oral, hemoabdomen, gastrointestinal, other). Owners were also instructed to contact investigators with any concerns.

Tapering of UFH

Heparin administration was discontinued once there were positive trends in the dog's hematocrit and leukogram. Specific criteria for discontinuation were a steadily increasing hematocrit (>25%) and resolution of the inflammatory leukogram (i.e., no metamyelocytes, no band neutrophilia, and a decreasing mature neutrophilia).

Heparin therapy was discontinued over a 3-day period by decreasing the frequency of administration. Heparin was administered every 8 hours the first day of the decreased dose, then every 12 hours the second day, and just once the final day of administration. Guidelines for when and how to discontinue UFH treatment were developed based on current clinical practice in the authors' hospital.

The time of each dog's discharge was at the discretion of the clinician. For all dogs discharged (n=15), UFH was provided for the owner to administer for a period of time at home. Dogs received the same dose and frequency of administration of UFH after discharge as during hospitalization. After discharge, dogs were monitored by frequent recheck visits, telephone contact with owners, and telephone contact with referring veterinarians.

Necropsy Examination

Owners agreed to necropsy examination if the dog were to die within 12 months of enrollment in this study. Necropsy examinations were conducted within 24 hours of the dog's death, and they were performed by the board-certified pathologist on duty at Purdue University Veterinary Teaching Hospital. Animal remains were kept refrigerated prior to necropsy examination. Histopathology was performed for all necropsied dogs.

Statistical Analysis

All data analysis was performed using Prizm statistical software.[1] Distribution for normality of continuous variables was checked using the Shapiro-Wilk test. Because of the number of variables with nonparametric distributions at individual time points and the small number of dogs, all summary statistics are expressed as median and range. The Friedman two-way analysis of variance by ranks was used for comparison of anti-Xa activity across four time points. Using the Wilcoxon signed rank test, pairwise comparisons of anti-Xa activity at two different time points, as well as comparisons of antithrombin activity at two time points, were made. Correlations between anti-Xa activity and aPTT for all dogs and time points were determined using Spearman rank correlation coefficient. A *P* value <0.05 was considered statistically significant.

Results

The 18 dogs in the study ranged in age from 5 months to 13 years (median 7 years). Twelve breeds were represented, including mixed-breed (n=4), American cocker spaniel (n=2), beagle (n=1), Chesapeake Bay retriever (n=1), dachshund (n=1), Doberman pinscher (n=1), English setter (n=1), Labrador retriever (n=2), Lhasa apso (n=1), Maltese (n=1), rottweiler (n=1), shih tzu (n=1), and Yorkshire terrier (n=1). Of the 18 dogs, 72% were female and 28% were male; 11 were spayed females, two were neutered males, two were intact females, and three were intact males.

Fifteen dogs were enrolled within 24 hours of diagnosis of IMHA. Immune-mediated hemolytic anemia had been diagnosed and treated with corticosteroids for 2 to 4 days (median 2 days) in the remaining three dogs. Hematocrit at presentation ranged from 9.5% to 22.5% (median 15%). Spherocytes were present in 12 (66%) dogs; spherocytes were rated 3+ in two dogs, 2+ in five dogs, 1+ in four dogs, and were noted occasionally in one dog. Sixteen (88%) dogs had a positive slide agglutination test. Agglutination was present in all dogs with <2+ spherocytes. Eleven dogs had a positive Coombs' test. Reticulocyte count on presentation ranged from 12.5 to 426 × $10^3/\mu$L in the 10 dogs for which it was determined (median 164 × $10^3/\mu$L). In eight dogs, reticulocyte count could not be determined because of agglutination, but reticulocyte percentage ranged from 0.3% to 25.7% (median 4.1%). The anemia was classified as nonregenerative in six dogs (absolute reticulocyte count <60 × $10^3/\mu$L or reticulocyte production index <2). Platelet counts ranged from 60 to 352 × $10^3/\mu$L (median 200 × $10^3/\mu$L). In seven dogs, platelet counts were not reported because of clumping. Six of these dogs were considered to have adequate numbers of platelets, and one was considered to have decreased platelets on visual inspection of the blood smear. Overall, six dogs had thrombocytopenia.

One dog received a transfusion of fresh whole blood (45 mL/kg) prior to study enrollment and did not receive any additional transfusions after enrollment. Five dogs did not receive transfusions during their initial hospitalization. Four dogs received a single transfusion of cross-matched compatible pRBCs; seven dogs received two transfusions; one dog received three transfusions; and another dog received five transfusions. The dog that received three RBC transfusions also received two transfusions with a hemoglobin-based oxygen carrier.[m]

Fourteen dogs received prednisone at a dosage of 2 mg/kg PO *q* 12 hours; three dogs received a lower prednisone dose at 20 mg/m^{2}; and one dog received dexamethasone (0.28 mg/kg IV *q* 12 hours) because of protracted vomiting. One dog had a prednisone dosage reduction on day 5 (to 1 mg/kg PO *q* 12 hours) because of suspected pancreatitis. At the discretion of the primary clinician, azathioprine therapy (2 mg/kg PO *q* 24 hours) was initiated in one dog while in the hospital (day 1), because a markedly elevated serum bilirubin of 23.6 mg/dL was considered a poor prognostic indicator. Two dogs had azathioprine therapy added after discharge (at week 2 and week 11) because of continued anemia and persistent agglutination (n=1) and the development of calcinosus cutis (n=1), respectively. The dog treated with dexamethasone also was given cyclophosphamide (50 mg/m^{2} IV *q* 24 hours) for 3 days (starting day 4), as four transfusions of pRBCs had been administered, and emesis prevented administration of azathioprine.

Pretreatment Coagulation Panel Results

Median aPTT was 14 seconds (range 11 to 90 seconds; reference interval 10 to 17 seconds). Fifteen dogs had normal aPTT, and three dogs had prolonged aPTT. Median PT was 17.3 seconds (range 14.5 to 27 seconds; reference interval 13 to 18 seconds). Seven dogs had PT values that were prolonged, and 11 dogs had PT values within the reference interval. All dogs had high D-dimer concentrations (250 to 500 ng/mL [n=3], 500 to 1000 ng/mL [n=4], 1000 to 2000 ng/mL [n=7], and >2000 ng/mL [n=4]; reference interval <250 ng/mL). All dogs had high fibrinogen concentrations (median 1090 mg/dL; range 562 to 1948 mg/dL; reference interval 147 to 479 mg/dL).

Antithrombin Activity

Median baseline antithrombin activity was 79% (range 47% to 109%; reference interval 75% to 120%). Eleven dogs had baseline antithrombin within the reference interval, and seven dogs had low antithrombin. Median antithrombin at 40 hours was 54% (range 18% to 101%). Four dogs had 40-hour antithrombin within the reference interval; 13 dogs had low antithrombin; and one dog died prior to that time point. The median antithrombin at 40 hours was significantly lower than the median baseline antithrombin (*P*=0.0018) [Figure 1]. Two (28.6%) of the seven dogs with low antithrombin activity at baseline attained anti-Xa activity within the target range. Three (27.3%) of 11 dogs with normal baseline antithrombin activity attained anti-Xa activity within the target range.

Anti-Xa Activity During the 40-Hour Intensive Monitoring Period

Unfractionated heparin was administered at an initial dosage of 300 IU/kg SC *q* 6 hours to all dogs for the first 40
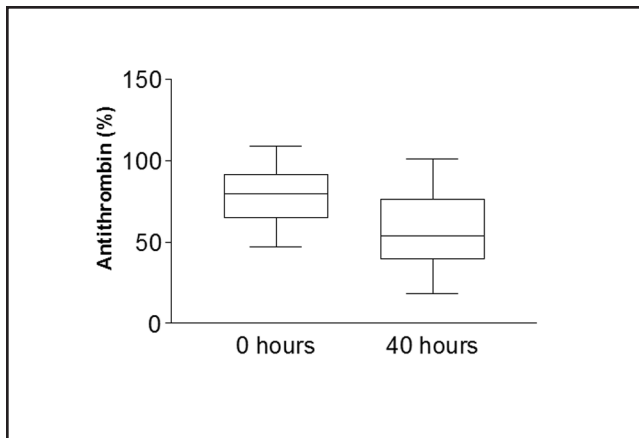
Figure 1—Box plot of antithrombin activity at 0 and 40 hours in dogs with primary immune-mediated hemolytic anemia administered unfractionated heparin subcutaneously (300 IU/kg *q* 6 hours). Boxes represent data in the interquartile range (IQR); the center lines represent the medians; and the whiskers indicate data observations within 1.5*IQR for the first and third quartile.

hours of treatment. One dog died prior to collection of the 40-hour sample. For another dog, anti-Xa activity could not be measured in the 28- and 40-hour samples, because high plasma hemoglobin (caused by administration of a hemoglobin-based oxygen carrier) interfered with assay endpoint determination. The anti-Xa activities for the remaining dogs at the four sample time points (i.e., 4 hours, 16 hours, 28 hours, 40 hours) are depicted in Figure 2. Compared to 4-hour measurements, median anti-Xa activity was significantly increased at 16 hours (*P*=0.002) and at 28 hours (*P*=0.014), but not at 40 hours (*P*=0.069). Median anti-Xa activity at 16 and 28 hours was significantly higher than the activity at 40 hours (*P*=0.048 and *P*=0.006, respectively). At the 4-hour time point, only one dog had attained the target

range of 0.35 to 0.70 U/mL. The number of dogs having values within the target range was five, six, and five at the 16-, 28-, and 40-hour samples, respectively. Two dogs had values above the target range at 16 hours, and one of these dogs remained above the target range at 28 hours. Both dogs had increases in the aPTT at the time that the anti-Xa activity was above the target range. Neither dog had an increase in aPTT prior to UFH treatment. The UFH dosage was decreased in one of these dogs prior to collection of the 28-hour sample, based on a prolonged in-house aPTT of 64.2 seconds. The anti-Xa activity for this dog was within the target range at the 28-hour sample but below the target range at the 40-hour sample. This dog was euthanized prior to a second dose adjustment.

Eight dogs achieved anti-Xa activity within or above the target range during the first 40 hours of UFH therapy, with seven of the eight dogs maintaining target range values for two or more time points. The highest measured plasma anti-Xa activity was 1.2 U/mL. None of the dogs developed signs of abnormal bleeding, bruising, or decline in hematocrit because of blood loss. Ten dogs had anti-Xa activity <0.35 U/mL throughout the initial 40-hour treatment period. Of the 16 dogs in which anti-Xa activity could be evaluated at 40 hours, five dogs were within target range and 11 were below target range. The relationship between measured aPTT and anti-Xa activity was not linear for raw data, but the natural logarithm (ln) of measured aPTT was linearly related and strongly correlated with anti-Xa activity (rho=0.765; *P*<0.0001) [Figure 3].

Adjustments in UFH Dosage

Four of the five dogs that were in the target range at 40 hours were continued on UFH at the same dosage, while in one dog the dosage was decreased based upon a 40-hour aPTT value that was prolonged (55 seconds). Of the 11 dogs that were below the target range at 40 hours, four dogs were tapered from UFH based on the criteria previously outlined,
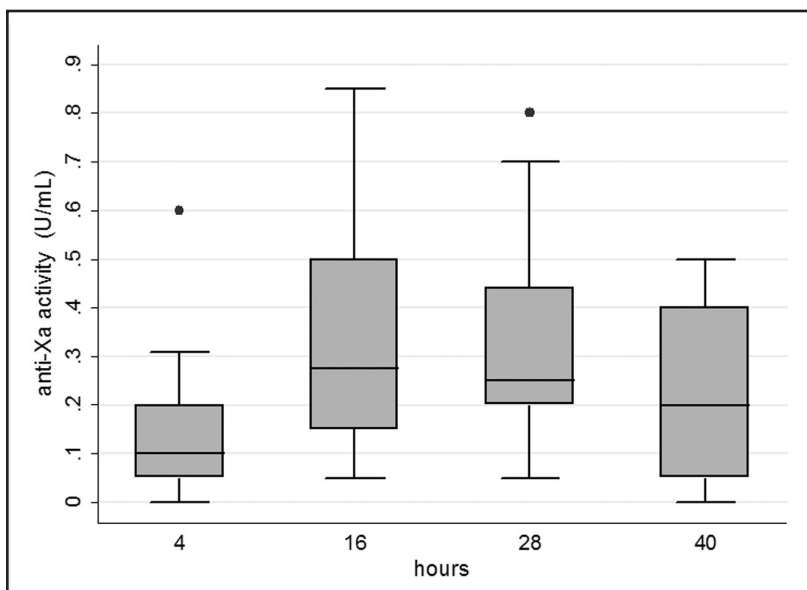
Figure 2—Box plot of anti-Xa activity at 4, 16, 28, and 40 hours in dogs with primary immune-mediated hemolytic anemia administered unfractionated heparin subcutaneously (300 IU/kg *q* 6 hours). Boxes represent data in the interquartile range (IQR); the center lines represent the medians; the whiskers indicate data observations within 1.5*IQR from the first and third quartile; and dots represent outliers.
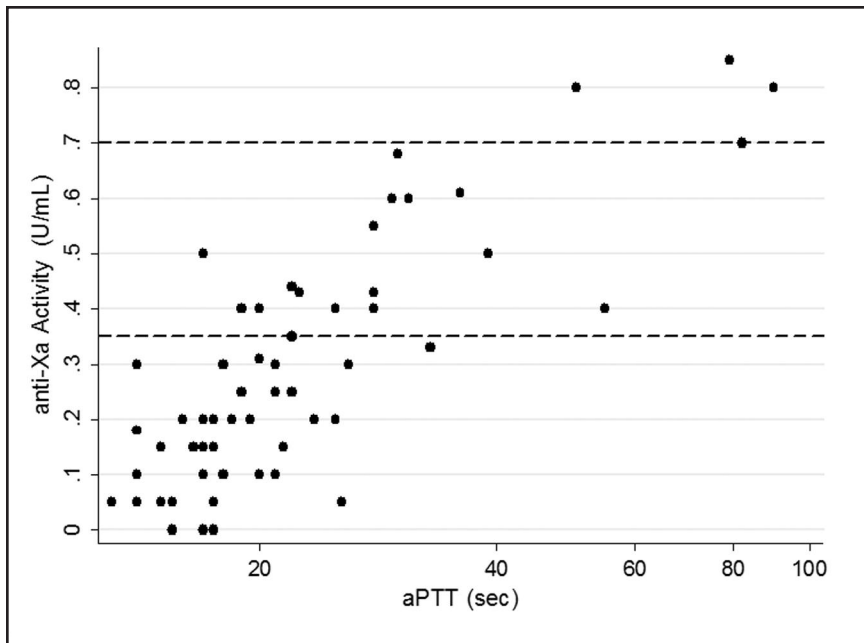
Figure 3—Relationship between aPTT and anti-Xa activity at four different time points (4, 16, 28, and 40 hours) in 18 dogs with primary immune-mediated hemolytic anemia administered unfractionated heparin subcutaneously (300 IU/kg *q* 6 hours) (rho=0.825; *P*<0.001). The aPTT axis is shown on log scale. Dashed lines represent the target range (0.35 to 0.70 U/mL) for anti-Xa activity.

while UFH dosage was increased in six dogs. One dog was euthanized prior to a dosage adjustment.

Anti-Xa Activity in Follow-up Period

Seven dogs did not receive UFH beyond the initial 40-hour monitoring period (four had been tapered from UFH therapy, and three had died or were euthanized at this point). The remaining 11 dogs continued UFH treatment after the first 40-hour monitoring period, and in these dogs UFH therapy was administered at home by the owners after discharge from the hospital. In the six dogs in which the UFH dose was increased, the increase ranged from 17% to 25% (300 to 350 or 375 units UFH), with a higher increment for dogs with no prolongation of aPTT or very low anti-Xa activity. Three additional dogs attained anti-Xa activity within or above target range after this dose adjustment. In two dogs, anti-Xa activity remained below the target range after dosage adjustment, and one dog was lost to follow-up. Two of four dogs that received the highest dosage of UFH (375 IU/kg SC *q* 6 hours) still did not attain anti-Xa >0.35 U/mL. Median duration of UFH treatment for all 18 dogs in the study was 14 days (range 1 to 23 days). Median duration of UFH treatment at home was 9 days (range 2 to 20 days).

Short-term and Long-term Survival

Fifteen (83%) of the 18 dogs survived to discharge. Thirteen (72%) dogs were alive 1 month after diagnosis, and 11 (61%) dogs were alive 1 year after diagnosis. The seven nonsurvivors at 1 year included five dogs that died (two during initial hospitalization and three after discharge) and two that were euthanized (one during initial hospitalization because of severe hemolysis and protracted emesis, and the other 9.5 weeks after discharge because of disseminated

fungal infection). The dog with the disseminated fungal infection had been treated with azathioprine and prednisone.

Thromboembolism

Necropsy was performed on six of the seven nonsurvivors, and thrombi were identified in three of these six dogs. One dog had an iliac arterial thrombus and died on day 4 of treatment while receiving UFH at 300 IU/kg *q* 6 hours. This dog's last measured anti-Xa activity was 0.33 U/mL at the 16-hour sample. Administration of hemoglobin solution interfered with anti-Xa analyses at the 28- and 40-hour samples. The second dog with necropsy-confirmed thrombosis had microthrombi within alveolar capillaries; this dog died 6 days after discharge while receiving UFH administered by the owner at 300 IU/kg. The last measured anti-Xa activity in this dog was 0.4 U/mL at the 40-hour sample. The third dog with confirmed thrombosis died 2 months after initial diagnosis; this dog had a mesenteric artery thrombus, portal vein thrombus, pulmonary artery thrombi, and hepatic thrombi. In the 6 weeks prior to this dog's death, no UFH had been administered. This dog's hematocrit had returned to normal, and the prednisone dosage had been decreased to 50% of the initial amount (10 mg/m^2 *q* 12 hours, 0.58 mg/kg *q* 12 hours) 2 weeks prior to death. In the remaining three nonsurvivors in which thrombi were not identified, one dog died and two were euthanized. In the dog that died, icterus, pulmonary edema, pericardial effusion (minimal), left ventricular hypertrophy, hepatic lipidosis, mild hydrocephalus, and ancyclostomiasis were noted at necropsy. In the two dogs that were euthanized, multifocal cutaneous blastomycosis and phaeohyphomycosis were noted in the first dog, and hemolytic anemia, hemoglobin nephrosis, necrotizing hepatitis, and hepatic passive congestion were

noted in the second dog. No secondary cause for IMHA was identified in any dog at necropsy.

Discussion

Most of the available pharmacokinetic data regarding UFH are derived from studies of healthy dogs. Kellerman and colleagues reported that in beagles, a single UFH dose of 250 IU/kg SC resulted in plasma anti-Xa activity in the range of 0.35 to 0.70 U/mL.[23] Subcutaneous administration every 6 hours was recommended based on anti-Xa activity remaining >0.35 U/mL for 5 to 6 hours postinjection.[23] In studies by Mischke *et al*, one UFH dose of 500 IU/kg SC to beagles resulted in mean anti-Xa activity of 0.4 U/mL; however, repeated doses of 500 IU/kg given *q* 8 or *q* 12 hours resulted in UFH activity >0.8 U/mL and hematoma formation in two of 10 dogs.[12] Diquélou *et al* reported that a single dose of 200 IU/kg UFH given SC to healthy dogs resulted in a 1.35- to 2.01-fold increase in aPTT and an anti-Xa of 0.42 to 0.9 U/mL.[13] In another study of healthy dogs, a UFH dose range of 250 to 500 IU/kg was found to achieve a 1.5- to 2.5-fold increase in aPTT.[26]

The pharmacokinetics of UFH in dogs with IMHA have not been described, and UFH monitoring is rarely reported in clinical studies. The empiric UFH dosage regimens used for IMHA vary widely, encompassing a range of 50 to 500 IU/kg *q* 6 to 12 hours.[3,9,27-29] Rozanski *et al* monitored prolongation of aPTT in response to 200 IU/kg UFH SC *q* 6 hours (with or without plasma) in 15 critically ill dogs, including six dogs with IMHA.[30] Of the two dogs with IMHA that received only UFH, one had a mild increase in aPTT, and one had a moderate increase in aPTT after 24 hours.[30] Of the four dogs with IMHA that received UFH and fresh-frozen plasma (10 to 15 mL/kg *q* 12 hours), two had stable aPTT values and two had a mild increase in aPTT 24 hours after starting treatment.[30] Anti-Xa activity was not measured in this study.

In a previous study of IMHA, 13 dogs were treated with UFH at a dosage of 100 IU/kg SC *q* 6 hours and a single dose of fresh-frozen plasma (10 mL/kg).[9] In this study, the mean anti-Xa activity after 24 hours was 0.22 U/mL, and after 48 hours it was 0.11 U/mL. Although 10 of 11 dogs had anti-Xa <0.35 U/mL at 24 hours, one dog had a value of 1.4 U/mL.[9] Ultimately, only one of 10 dogs evaluated attained anti-Xa activity in the range of 0.35 to 0.70 U/mL at the 48-hour time point.[9]

Because of the variable pharmacokinetic profile of UFH, individual monitoring is necessary.[15,17] In this study, a UFH dosage of 300 IU/kg *q* 6 hours was generally inadequate to attain anti-Xa activity >0.35 U/mL. Only eight of 18 dogs attained target-range anti-Xa activity at any time using this dosage. These results indicate that attainment of the predetermined target range by 40 hours will require a more aggressive and individualized dosage regimen. The requirement for higher dosages of UFH in dogs with IMHA is not unexpected. Heparin binds to many plasma proteins, and induction of an acute-phase response further increases the concentration of UFH-binding proteins.[31,32]

All of the dogs in this study had marked elevation of plasma fibrinogen at admission, consistent with an active inflammatory state. Heparin binding to activated endothelial cells and macrophages may further contribute to low anti-Xa activity in this disease population. Initiation of higher dosages of UFH (>200 IU/kg SC *q* 6 hours) seems warranted in dogs with IMHA to obtain sufficient anticoagulant intensity for the desired thromboprophylactic effect. Higher UFH dosages, however, potentially increase the risk of UFH-associated bleeding and other possible complications of UFH therapy. While heparin-induced thrombocytopenia (HIT) is a common adverse drug reaction in humans, the phenomenon has not been reported in dogs.[33] Less commonly encountered adverse reactions that have been reported in humans include vasospastic reactions, osteoporosis and diminished renal function, rebound hyperlipidemia, hyperkalemia, alopecia, suppressed aldosterone synthesis, and priapism.[17,34,35] Although many of the dogs in this study were thrombocytopenic prior to institution of UFH therapy, none of them experienced clinically detectable bleeding tendencies. Possible causes of progressive thrombocytopenia (e.g., Evans' syndrome, HIT, disseminated intravascular coagulation) in IMHA dogs may be difficult to differentiate. Necropsy evaluation of the dogs that died showed no hemorrhage that could be attributed to UFH treatment. Based on these results, administration of UFH at an initial dosage of 300 IU/kg *q* 6 hours appears safe, if carried out in conjunction with appropriate laboratory monitoring.

Heparin's anticoagulant effect is most often detected by prolongation of aPTT, which is a reflection of the UFH-antithrombin complex's inhibition of thrombin, factor Xa, and other serine protease coagulation factors.[36] Prolongation of aPTT, however, is not a specific measure of UFH effect, and excessive UFH dosages may be required to prolong aPTT in certain inflammatory syndromes.[15,37] Different aPTT reagents have highly variable sensitivity to UFH, further complicating establishment of uniform treatment guidelines and precluding comparisons among treatment trials. Recently, anti-Xa activity assays have been used to monitor and adjust UFH dosage and to calibrate aPTT assays to attain target UFH levels.[14,15,38] In this study, dogs were monitored by both aPTT and anti-Xa activity assays. In most dogs, anti-Xa activity was used to determine dosage adjustments, but in some dogs a prolonged in-house aPTT mandated decreasing the UFH dosage prior to knowledge of the anti-Xa activity. For this reason, it is difficult to assess whether reliance on the anti-Xa activity alone would have been adequate to predict a bleeding tendency. Further investigation into the ideal method of using aPTT and anti-Xa assays to monitor UFH therapy are necessary. Anti-Xa assays can also be used to detect the anticoagulant effect of low molecular-weight heparins and allow direct comparisons of UFH to newer drugs.

The authors' study was not designed to test the efficacy or validate a target range of UFH for thromboprophylaxis of IMHA. Its limitations include small sample size and lack of controls. The observed survival to discharge rate of 15 of 18

dogs and 1-year survival rate of 11 of 18 dogs meet or exceed those of other prospective and retrospective studies of IMHA.[5,9,29,39-42] Direct comparisons of survival between studies cannot be made, however, because of variations in study populations, treatment regimens, and factors influencing euthanasia rates. Since thromboembolism is a major cause of mortality in dogs with IMHA, a rigorous examination of UFH (and low molecular-weight heparins) for thromboprophylaxis is warranted. While dosage adjustments in this study were based on an anti-Xa target range of 0.35 to 0.70 U/mL, this target may not be optimal for IMHA. Two of the three dogs with necropsy-confirmed thrombi had anti-Xa values within the target range at the last measured time point; however, the interval from UFH monitoring to death ranged from 2 to 4 days.

Another issue raised by the authors' study is the length of time that UFH treatment should be continued. Heparin therapy was empirically discontinued in the dogs of this study based on clinical status (i.e., rising hematocrit, resolution of inflammatory leukogram); however, one dog had a thromboembolic event long after normalization of hematocrit. This dog died from mesenteric thrombosis 2 months after diagnosis of IMHA, and jaundice was noted at necropsy. The most likely reason for thrombosis in this dog was continued hemolysis related to IMHA, but steroid administration could have contributed as well. Long-term anticoagulation may be necessary in at least some dogs with IMHA.

Conclusion

Dogs in this study were relatively resistant to UFH at a dosage of 300 IU/kg *q* 6 hours, based on *in vitro* measures of its anticoagulant effect. Initiation of UFH at a dosage of 300 IU/kg *q* 6 hours was inadequate to attain an anti-Xa activity >0.35 U/mL within the first 2 days of therapy for 10 of 18 dogs with IMHA. Controlled, multicenter prospective studies are warranted to determine whether a dosage schedule of UFH, low molecular-weight heparins, or aspirin can be developed to provide effective thromboprophylaxis in dogs with IMHA.

Footnotes

[a] Canine Coombs' Reagent; VMRD, Inc., Diagnostics, Pullman, WA 99163
[b] Heparin sodium injection; Elkins Sinn, Inc., Cherry Hill, NJ 08003
[c] Dade Actin Reagent; Baxter Diagnostics, Edison, NJ 08003
[d] Dade Actin FS Reagent; Baxter Diagnostics, Edison, NJ 08003
[e] Thromboplastin LI; Helena Diagnostics, Beaumont, TX 77704
[f] Fibrinogen; Diagnostica Stago, Parsippany, NJ 07054
[g] STA Compact; Diagnostica Stago, Parsippany, NJ 07054
[h] D-dimer latex; Biopool, Wicklow, Ireland
[i] Stachrom ATIII; Diagnostica Stago, Parsippany, NJ 07054
[j] Rotachrom Heparin; Diagnostica Stago, Parsippany, NJ 07054
[k] STA Rotachrom Heparin package insert; Diagnostica Stago, Parsippany, NJ 07054
[l] GraphPad Prism; Graphpad Software, Inc., San Diego, CA 92130
[m] Oxyglobin Solution; Biopure Corporation, Cambridge, MA 02141

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